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Feasibility of Biodegrading TNT-Contaminated Soils in a Slurry Reactor

by C.D. Montemagno and R.L. Irvine

Environmental Assessment and Information Sciences Division
Argonne National Laboratory, Argonne, Illinois 60439-4801

June 1990 *(Final Report)*

prepared for

Commander
U.S. Army Toxic and Hazardous Materials Agency
Aberdeen Proving Ground, Maryland 21010-5401

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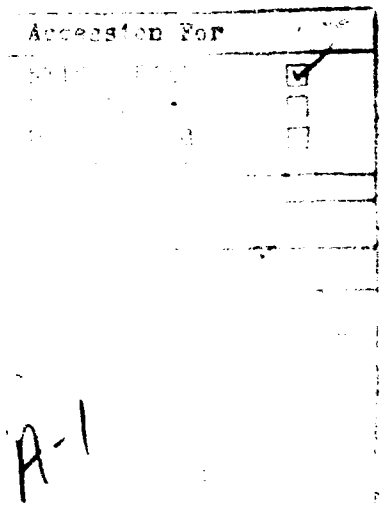

FEASIBILITY OF BIODEGRADING TNT-CONTAMINATED SOILS IN A SLURRY REACTOR

by

C.D. Montemagno and R.L. Irvine

ABSTRACT

This report presents the results of a study of the feasibility of treating explosives-contaminated soils through biodegradation by bacteria. Soil samples were collected from the Joliet Army Ammunition Plant, and a bacterial consortium tolerant to trinitrotoluene (TNT) was isolated for bench-scale testing in a soil-slurry reaction system. Initial experiments indicated that the consortium can use TNT as a source of carbon, nitrogen, or both. Additional experiments determined system conditions (e.g., type and quantity of nutrients) that enhanced TNT consumption by the consortium. The study results indicate that a soil-slurry/sequencing-batch reactor merits testing as an on-site, pilot-scale system. This report also presents a pilot-scale design and cost analysis.

1 INTRODUCTION

Historical accounts of explosives go back to ancient Chinese and Greek cultures. Gunpowder was developed in the west during the 13th century, military use of explosives began in the 14th century, and commercial use began in the early 17th century. Gunpowder is a well-ground mixture of potassium nitrate, charcoal, and sulfur.[1] The manufacture of gunpowder for munitions in North America began in the 17th century.[2]

High explosives came into existence in the middle of the 19th century. The term "high explosive" refers to detonation initiated and maintained by shock waves rather than rapid burning, as in the case of gunpowder. In either case, the explosion is caused by the rapid conversion of solid or liquid into many times its volume as gas. High explosives will not ignite and require an initiating explosive for detonation. In general, explosives fall into one of three categories: propellent, primary (initiating), and secondary (high explosive). Explosives generally contain enough oxygen, usually in the form of nitro groups or nitrate, to completely combust in the absence of external oxygen.[1]

Nitroglycerin, the first high explosive, was developed by Sobero in 1847. Trinitrotoluene (TNT) was developed by Wilbrand in 1863. Alfred Nobel went on to perfect the manufacture of explosives.[1] World Wars I and II brought about the development of many large munitions plants in the United States. Many more facilities

provided for the repacking of spent or dated munitions. The manufacture of explosives and the cleaning and the repacking of old munitions requires large quantities of water, which became contaminated with explosives during processing. For years, explosives wastewater (pink water) was discarded outside of the manufacturing facilities, on the ground or in lagoons that leached explosives into the soil, as well as into groundwater, rivers, and lakes. These compounds are quite recalcitrant (slow to degrade) and highly toxic. Some breakdown by-products are even more toxic than the original material.

As a result of historic explosive manufacturing and storage, large volumes of soil contaminated with TNT and related compounds (e.g., 1,3,5-trinitro-1,3,5-triazine [RDX]) are present at numerous federal facilities. The costs associated with the remediation of these sites have been estimated to be in excess of $1.5 billion. Studies sponsored by the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) have explored both composting and land farming techniques and have validated the concept of biologically decontaminating TNT-laden soils using composting. This study was directed at the development of microbial based design concepts for soil-slurry/sequencing-batch reactors (SS/SBRs), in which explosives-contaminated soils are mixed with water (i.e., slurried) and nutrients. The design provides for the complete distribution of the target compounds, biomass, nutrients, and oxygen while diluting highly contaminated areas of the soil. The design approach is similar to the periodic activated sludge processes that have been developed at the University of Notre Dame.[3] These periodic processes were successfully used by Bell, Burrows, and Carrazza to break down explosives.[4] In their study, up to 98% of the TNT present in a wastewater contaminated by explosives was degraded in a 4-h cycle.

Composting of explosives has proven to be effective, and half lives for the breakdown of TNT have ranged from 7 to 22 d.[5] Because of the large quantities of additives (e.g., straw, animal feed, etc.) that are used in composting, only a small fraction of the total volume composted is contaminated soil. The additives must be transported to the site and increase the final volume. The major additional component for the SS/SBR treatment system described herein is water, which would be provided from on-site wells or local supplies. After treatment, the water is easily removed in drying beds, leaving just the treated soil.

The current method of remediation for explosives-contaminated soils is incineration.[6] This is a costly, energy-intensive process that destroys much of the soil, leaving ash as the primary residue. SS/SBR remediation would return the soil undamaged to the original source. In addition, the returned soil will contain the biomass necessary to continue the breakdown of the explosives remaining at trace levels after initial treatment.

This report is divided into three parts: a literature review, a description of the TNT biodegradation studies, and a design and cost analysis of the SS/SBR bioremediation system developed. Results from this work demonstrate that (1) TNT can be mineralized (broken down under aerobic conditions to produce carbon dioxide [CO_2]) using a bacteria-based microbial consortium isolated from soils from the Joliet Army Ammunition Plant (JAAP) and (2) TNT-contaminated soils can be bioremediated in the SS/SBR system at a cost that ranges between $30 and $150/yd^3, depending upon the quantity of soil treated and the overall rate of TNT destruction.

Suggested future studies include the characterization of the metabolic activities of the consortium responsible for degrading TNT; isolation of specific metabolites to define the pathway of TNT destruction; investigation of the limiting factors affecting TNT metabolism, including characterizing the involvement of cometabolism; isolation of the genes and enzymes responsible for the TNT metabolism; investigation of molecular techniques such as site-directed mutagenesis, which will further enhance the rate of TNT destruction; investigation of the biodegradation of RDX and HMX; determination of the toxicity level of either any intermediates produced during bioremediation or any contaminants not degraded during treatment; investigation of the breakdown of TNT by the white-rot fungus, *Phanerochaete chrysosporium*, in a fixed-film reactor; operation of bench-scale soil slurry reactors to further quantify the pilot- and full-scale designs; and design and construction of a pilot plant for operation at the JAAP.

2 LITERATURE REVIEW

Biological removal of explosives from wastewater[4] indicates that the proper conditions to obtain biological breakdown of the explosives in contaminated soils may be achievable in soil-water slurries. A detailed literature review was directed at how a microbial-based consortium can be used to degrade explosives. The search used the following keyword list:

TNT: 2,4,6-trinitrotoluene (alpha symmetrical form)

DNT: 2,4-dinitrotoluene

RDX: hexahydro-1,3,5-trinitrotoluene-1,3,5-triazine

HMX: octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine

SEX: octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine

TAX: hexahydro-1-acetyl-3,5-dinitro-1,3,5-triazine

Tetryl

Nitrocellulose

Pink water

Biodegradation

Microbial

Degradation

Denitrification

Results from this search are provided below.

Turkeltaub and Wiehl reported on the use of soil incineration to remove the by-products of explosives production.[6] They claim to have completed decontamination at the Cornhusker Ammunition Plant, Grand Island, Nebraska, where 40,000 tons of soil were incinerated. They reported that incineration is in process at the Shreveport Louisiana Army Ammunition Plant.

Researchers from Los Alamos National Laboratory, New Mexico, indicated that using bacteria that consume nitroglycerin and TNT may be the most economical way to clean up sites contaminated with these compounds.[7] Microorganisms that seem to have a tolerance for explosives-contaminated soil and water have been found near some

munitions plants. The researchers have found a microorganism that can break down nitroglycerin, and now are trying to extend the work to TNT. Eventually, they want to apply their knowledge of explosives to similar compounds such as chlorinated solvents.

Stoichiometric reduction of aromatic nitro substituents to amino groups was demonstrated with trinitrotoluene in crude cell extracts of *Veillonella alcalesens* with molecular hydrogen as a reductant.[8] The study demonstrated that anaerobic organisms were able to perform an initial transformation of TNT; however, evidence for further breakdown was not given. The amino-substituted by-products of TNT breakdown have been demonstrated to be highly recalcitrant and toxic.[9]

Weston found that land farming is inadequate for biodegrading TNT, DNT, RDX, and HMX and removing them from the environment.[10] TNT is only modified, and cometabolic conditions are required for modification to occur at a significant rate. DNT is biotransformed, but the final products have not been conclusively determined. It is possible that both CO_2 and stable intermediates are formed, with the amount of each depending on the specifics of the system (microflora, physiochemical conditions, environmental matrix, etc.). RDX is biotransformed under anaerobic, cometabolic conditions. Once, again metabolites are produced. Partial HMX biodegradation has been observed under optimized (anaerobic) treatment conditions, but in general, HMX biodegradability is less than that of RDX.

Individually, the biodegradability of explosive compounds is generally low. In combination, their biodegradability is likely to be reduced. An individual pink water component may prove toxic to a segment of the microflora active against another component. In addition, it is especially unlikely that one biological treatment system can optimize the degradation of each of the pink water components. A variety of metabolites are produced from each of the four compounds. Toxicity concerns exist regarding these intermediates, as well as the parent compounds from which they arise.[10]

Traxler demonstrated ring cleavage of TNT, but the amount of ring cleavage was small.[11] Most studies of microbial TNT transformation were unable to demonstrate ring cleavage.

Another problem, which Kaplan and Kaplan attempted to solve using surfactants and polar and nonpolar solvents, was the fact that several TNT degradation products are bound up in the soil, making them very difficult to recover.[12,13] Equimolar amounts of surfactants were required to remove the TNT by-products from the soil, and the surfactant complexes turned out to be more toxic than the original products. The soil-binding properties of TNT by-products should be taken into account when attempts are made to biodegrade these compounds and monitor for their presence.

Enzinger acclimated sewage treatment microbes to TNT. When cultured in a nutrient broth (trypticase soy), these microbes decreased TNT from 100 to 1.25 ppm in 5 d.[14]

Won provided the first evidence that microbes could use TNT as a sole carbon source by demonstrating that three Pseudomonad-like organisms could oxidize TNT.[15] Sediment and aquatic TNT enrichment cultures were able to degrade TNT in a basal salts

medium, but the system required the addition of glucose or nitrogenous substances (yeast extract) for accelerated transformation. In a medium supplemented with 0.5% yeast extract, 80 mg/L of TNT was completely broken down.

Traxler et al. found a number of gram-negative bacteria from various sources that were able to use TNT as a sole source of carbon and nitrogen.[11,16] They also determined that yeast extract (100 ug/mL) stimulated TNT consumption. They found that 62% of the initial TNT was removed from a yeast extract-supplemented medium in 20 h. Nitrate was detected in the medium, indicating that nitro groups were removed from the ring. When the authors performed ^{14}C-TNT* studies, they detected the incorporation of TNT into the cellular material of two isolates. They also detected $^{14}CO_2$ and concluded that ring cleavage occurred. The amount of ^{14}C detected as $^{14}CO_2$ represented a very low percentage of the initial amount added (0.3-1.2%). The authors conducted studies indicating that the fixation of $^{14}CO_2$ by cells metabolizing TNT accounted for this low amount of free $^{14}CO_2$.

Naumora isolated a *Pseudomonas denitrificans* strain from soil polluted with industrial waste. The isolate transformed TNT concentrations from 100 mg/L to reduced, nitrogen-free metabolites in 4 d.[17]

Bell found that, in semicontinuous activated-sludge treatment systems, no significant TNT reduction was seen in anoxic conditions when the TNT concentration fell below 5 mg/L.[2] This was discovered through bench- and pilot-scale studies of a semicontinuous activated sludge wastewater treatment system proposed for cleaning wastewater from the Holston Army Ammunition Plant. The bench-scale model was run for 30 mo and the pilot model was run for 6 mo. These systems used both aerobic and anaerobic conditions. The rate of TNT removal was a function of available (biodegradable) chemical oxygen demand (COD), with the rate of removal being reduced to extremely low levels or ceasing when COD was exhausted. TNT removal was to below detectable levels. Much of the RDX and TAX was removed. SEX and HMX were only partially removed.

Spanggord found that 2,4-DNT was readily transformed by natural water microbes, with greater than 90% of a 10-ppm solution transformed in 6 d.[18] In marked contrast to what these investigations found with TNT, they found that the ring of ^{14}C-2,4-DNT could be cleaved. After 7 d, $^{14}CO_2$ accounted for 59% of the added ^{14}C.

Davis et al. used industrial seed organisms (undefined) and investigated the degradation of 2,4-DNT.[19] After 2 d, 50-10 mg/L of DNT was degraded, but no change was seen at 7 d.

Soli detected the disappearance of RDX when it was incubated anaerobically with purple photosynthetic bacteria.[20] Approximately 97% of the RDX (20 mg/L) was transformed after 5 d of incubation. It was hypothesized that the strongly reducing conditions of the photosynthetic culture were responsible for destruction of the RDX

*TNT radioactively labeled with carbon-14 (^{14}C).

molecule. Spanggord demonstrated that RDX breakdown required cometabolism and anaerobic conditions.[18]

McCormick found that some percentage of HMX and acetylated HMX remained unaltered after passage through a full-scale anaerobic treatment system.[21] Altering the average retention time of the system and/or providing supplemental nutrients may increase HMX removal.

Doyle found that wood chips treated with sewage sludge were very effective in the breakdown of several explosives-contaminated soils in bench-scale experiments.[22] However, when taken to the pilot scale, hay-horsefeed and manure composts were very successful, while sewage sludge was not. Their recommendation was to use hay-horsefeed or manure composting. The study used what appears to be good analytical procedures, and the results may indicate a better source of microorganisms for biodegradation.

Walsh conducted a very detailed literature review (56 references) to recommend analytical approaches for determining metabolization of explosives.[23] Many metabolic pathways are covered, in particular for TNT and similar explosives. One point that becomes apparent is that complete pathways for the breakdown of several explosives have been established. The review concludes that, while there are analytical procedures available for many of the explosives and their by-products, there are no standards for these compounds. If standards could be obtained, the procedures could be validated and used to assess the degree of environmental contamination.

3 TNT BIODEGRADATION STUDIES

Ten soil samples were collected from the JAAP and analyzed by Argonne National Laboratory (ANL) using the U.S. Army extraction procedure and high-performance liquid chromatography (HPLC), Method LWO2 (Appendix A). Results from these analyses are provided in Table 1. These soils were used for both the basic microbiological studies and the bench-scale reactor studies. The microbiological studies were directed at measuring (1) the enumeration, growth potential, and enrichment of bacteria present in the soil samples, (2) the uptake of ^{14}C-TNT by a consortium enriched from the soils, and (3) the conversion of ^{14}C-TNT to CO_2 by the enriched consortium in both liquid culture and soil slurries. The bench-scale reactor studies were directed at the assessment of the biological disappearance of TNT in (1) a 4-L glass-kettle slurry reactor, (2) a 10.9-L stainless-steel slurry reactor, (3) a series of 1-L reactors, and (4) a fixed-film reactor containing the white-rot fungus, *Phanerochaete chrysosporium*. Each of these studies is described in detail below and in Appendix B.

3.1 MICROBIOLOGICAL STUDIES

3.1.1 Enumeration, Growth Potential, and Enrichment

The number of bacterial colony forming units (CFU) per gram of soil was determined for nine of the ten samples collected from the JAAP (Table 1) by running samples in 10% soil slurries (10 g soil/100 mL Stanier's medium[24]) with aeration. At 1 h and again at 6 d, each slurry was serially diluted and the dilutions plated on enriched agar plates. Plates were counted for colonies after 4-6 d of incubation. The results are presented in Fig. 1.

The count for the sample from the production line deluge tank (TPDT) was among the highest observed (in excess of 10^7 CFU/g), even though this sample had the highest level (about 14%) of TNT. Most of the other samples contained reasonable bacterial numbers, ranging from 10^4 to 10^6 CFU/g. As can be seen in Fig. 1, the bacterial numbers increased by at least a factor of 10 over a 6-d period for virtually all samples. The soil samples were then analyzed for bacterial growth potential, the ability of the bacteria to grow on the organic carbon present in the soil. This was done by creating an aerated 10% soil slurry of each sample, making serial dilutions of each sample, and plating every 6 d (as above). The three samples with the highest concentrations of TNT were periodically enriched by adding phosphorous and nitrogen and plated every 3 d to follow growth patterns more closely. The colony counts for these three samples over a 10-d period are shown in Fig. 2. These results indicate that the bacteria present at the TPDT site can at least tolerate, if not degrade, TNT.

After the growth potential experiments, a number of enrichments were started that were designed to enhance any microbial TNT-degrading activity. Anaerobic enrichments tested for bacterial use of TNT as a sole nitrogen source,[25] as a sole nitrogen-carbon source,[26] as a sole nitrogen source with succinate present, and as an

TABLE 1 Results of TNT Analysis for JAAP Soil Samples

Sample Description	Abbre-viation	Concentration (ppm)
TNT production line deluge tank	TPDT	144,000
Washout #2	G6W2[a]	35,000 40,000
Washout #1 edge	WO1E	130
Red lagoon water runoff edge	RLWR	140
Washout #2 sidewall	WO2S	40
Red lagoon dry #2	RLD2	15
8R interface washout #2	W28V	2
Red lagoon dry #1	RLD1	NA[b]
Red water lagoon water	RWLW	NA
Washout #1	G6W1	NA

[a]Sample not plated for microbial analysis.

[b]Not analyzed.

alternative carbon source (with succinate used as the initial carbon source). Aerobic enrichments were similarly designed, with Stanier's media manipulated to achieve the desired conditions. The enrichments were monitored microbiologically (plating serial dilutions) as well as turbidometrically (monitoring changes in turbidity with a spectrophotometer). Two soil samples showed the most promise for TNT degraders: from the red lagoon water runoff edge (RLWR) and washout #1 edge (WO1E). The enrichments have resulted in two consortia of aerobic bacteria that appear to be able to modify TNT, but only in the presence of succinate.

HPLC analysis (using the U.S. Army Method LWO2) of a medium with a consortium growing in the presence of TNT and succinate shows the appearance of four chromatogram peaks with time. The TNT peak diminishes as they grow. The nature of these peaks will be analyzed in future experiments by gas chromatography/mass spectrometry (GC/MS) to identify the intermediates being produced from TNT. At this

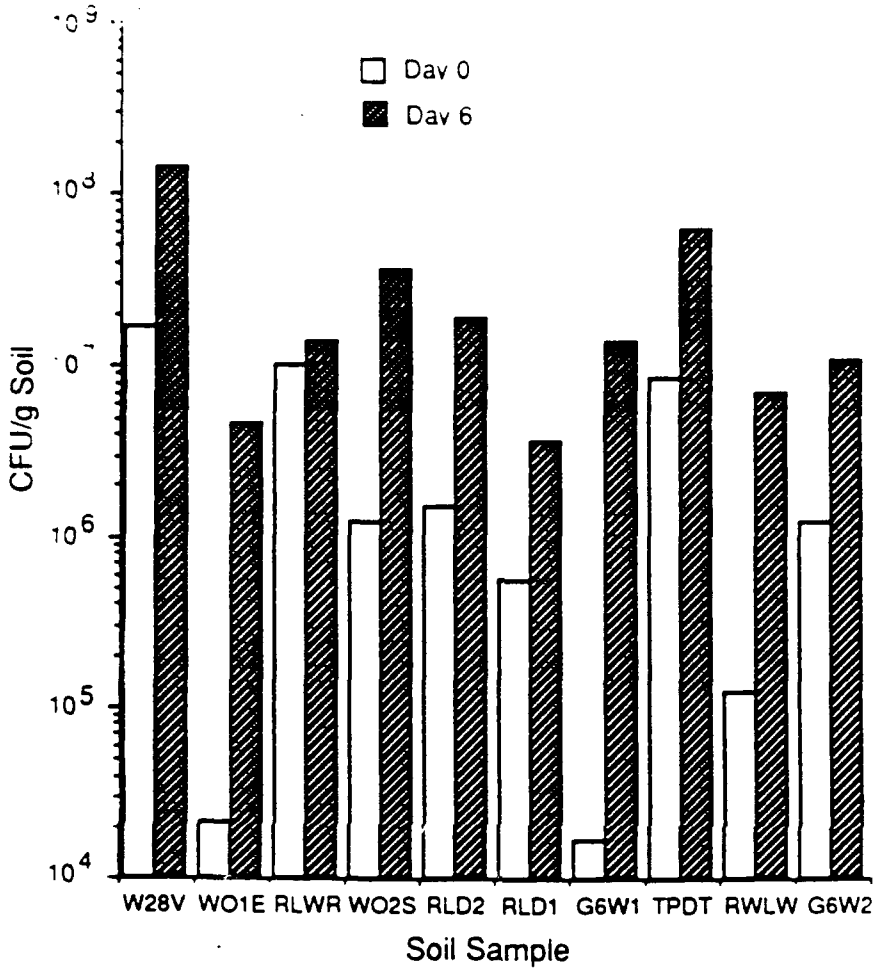

FIGURE 1 Colony Counts for JAAP Soil Samples

time, the intermediates are not believed to be the same intermediates observed by Kaplan and Kaplan.[9] This conclusion is based upon the reported mobilities of the Kaplan intermediates during HPLC analysis and the position of the peaks observed in this study. As described below, experiments with radiolabeled TNT were performed to determine whether any of the TNT was being metabolized. The need for an alternative carbon source such as succinate was also evaluated.

3.1.2 Metabolization of TNT

The ability of the RLWR consortium to metabolize TNT was evaluated with ^{14}C-TNT. The first experiment was designed to determine whether any of the TNT was being metabolized by the RLWR consortium. Succinate, when present in the reaction flask, was added at 0.1%. Aeration was provided by shaking. The total counts per minute (CPM -- as measured by a scintillation counter) per milliliter of reaction mixture (i.e., biomass plus supernatant) are plotted as a function of time in Fig. 3. As can be seen, the total counts are noticeably reduced in the flask containing the RLWR

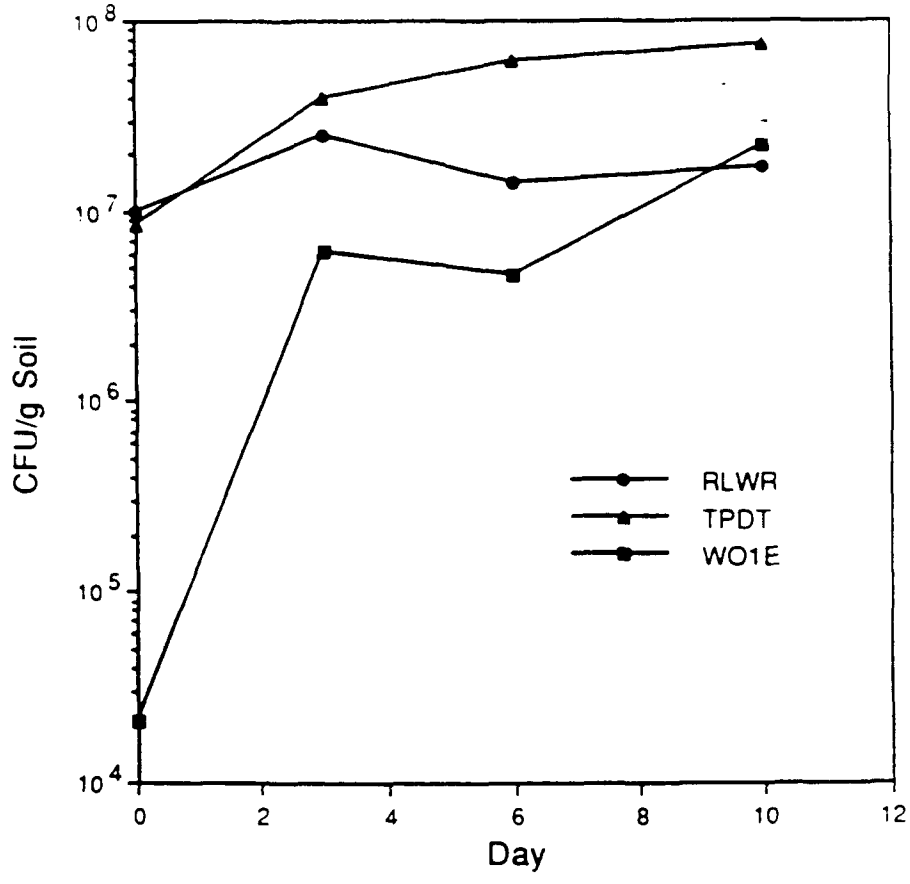

FIGURE 2 Microbial Growth Analysis of Selected JAAP Soil Samples

consortium culture and succinate. These results imply that TNT was being metabolized, possibly to CO_2, by the RLWR consortium.

The next experiment was designed to test the effects of succinate and cellular components on bacterial degradation of TNT. The results of this experiment are shown in Fig. 4. Each flask contained Stanier's medium, 100 ppm TNT, ^{14}C-TNT, and the RLWR consortium. Succinate, when present, was added at 0.1%. Conversion of TNT into biomass was determined by measuring the amount of ^{14}C-TNT converted into material precipitable by trichloroacetic acid (TCA). To do this, samples were withdrawn from the flasks and centrifuged with 20% TCA. The resulting pellet was washed and centrifuged with 5% TCA twice, and finally washed with a 50% ethanol-50% ether solution. The final pellet was resuspended in scintillation fluid, and radioactivity was determined with a scintillation counter. TCA-precipitable material is defined as macromolecular, and any radiolabel found in that fraction would only result from the metabolism of ^{14}C-TNT into biomass by the consortium. The data in Fig. 4 clearly show the conversion of TNT into cell mass by the RLWR consortium in the presence of succinate. Succinate appears to be required for metabolism, and conversion of TNT to biomass occurs only when active

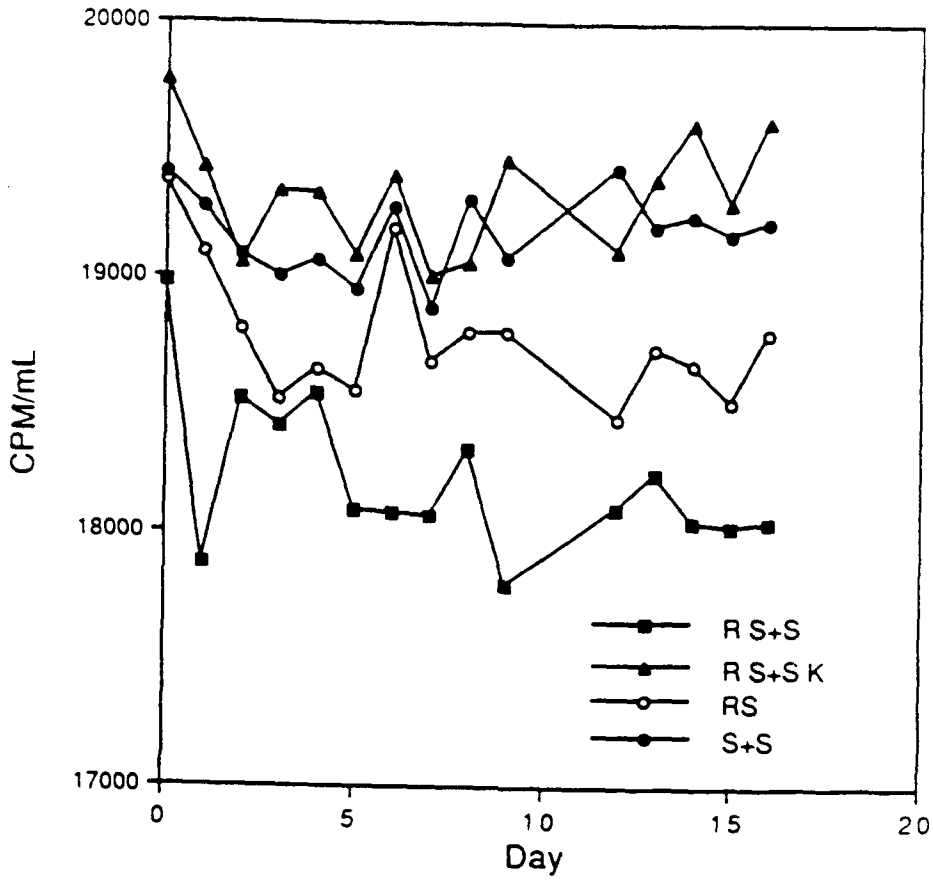

FIGURE 3 Assessment of RLWR Consortium Effect on Total TNT Culture (Key to flask designations: R S+S = RLWR consortium in Stanier's medium + succinate + 100 ppm ^{14}C-TNT, R S+S K = same as R S+S but slurry autoclaved to kill cells, RS = RLWR consortium in Stanier's medium + 100 ppm ^{14}C-TNT [no succinate], and S+S = Stanier's medium + succinate + 100 ppm ^{14}C-TNT [no consortium].)

bacterial cells are present. The total amount of conversion represents approximately 20% of the TNT in the flask. Thus, the RLWR consortium appears to be able to mineralize TNT to some extent, but not completely within the time frame of this experiment.

Figure 5 shows data from another experiment regarding the conversion of TNT to biomass by the RLWR consortium. In this experiment, run in duplicate, we manipulated the amount of succinate added to each flask. One flask received 0.1% succinate. The other two flasks received 0.1% succinate initially, but were provided with 10 uL and 20 uL of a 10% solution of succinate every 3 d. All flasks contained Stanier's medium, 100 ppm TNT, ^{14}C-TNT, and the RLWR consortium. The conversion to biomass was measured using the TCA method described above. As can be seen, the TCA-precipitable material steadily increased with time, indicating the accumulation of TNT metabolites in the biomass. The reason for the periodic reduction in TCA-precipitable material shown

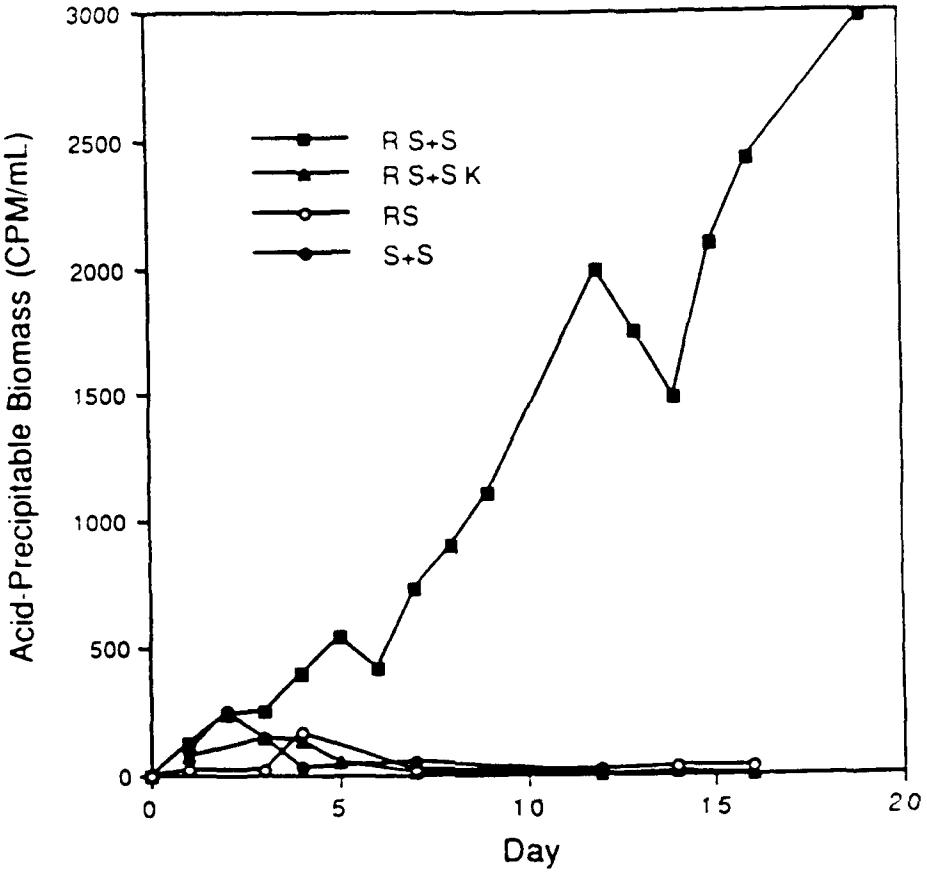

FIGURE 4 Conversion of ^{14}C-TNT to Biomass (see Fig. 3 for key to flask designations)

in Fig. 5 is not clear at this time but may result from the production of CO_2. Also, the addition of succinate repeatedly over the course of the experiment did not have a significant effect until after day 22. The data in Fig. 4 showed that succinate was required to initiate the metabolism of TNT. The data in Fig. 5 show that succinate may only be necessary to start the process and further additions may only be required at long intervals.

3.1.3 *Conversion of* TNT to Carbon Dioxide

Since TNT is being metabolized into biomass, it is important to see if any of the TNT is being mineralized to CO_2. Respirometers developed by Bartha and Pramer were used to determine conversion to CO_2.[27] The RLWR consortium, Stanier's medium, ^{14}C-TNT, and 0.1% succinate were placed in the respirometer. The CO_2 evolved and was passively trapped by potassium hydroxide (KOH), which was sampled, replaced, and counted for radioactivity every 2 d. Any radioactive CO_2 evolved was assumed to arise from ^{14}C-TNT. Controls for the experiment involved the killing of the consortium by autoclaving or eliminating either succinate or the consortium from the materials added. Figure 6 shows the results of this experiment. CO_2 is being evolved from TNT,

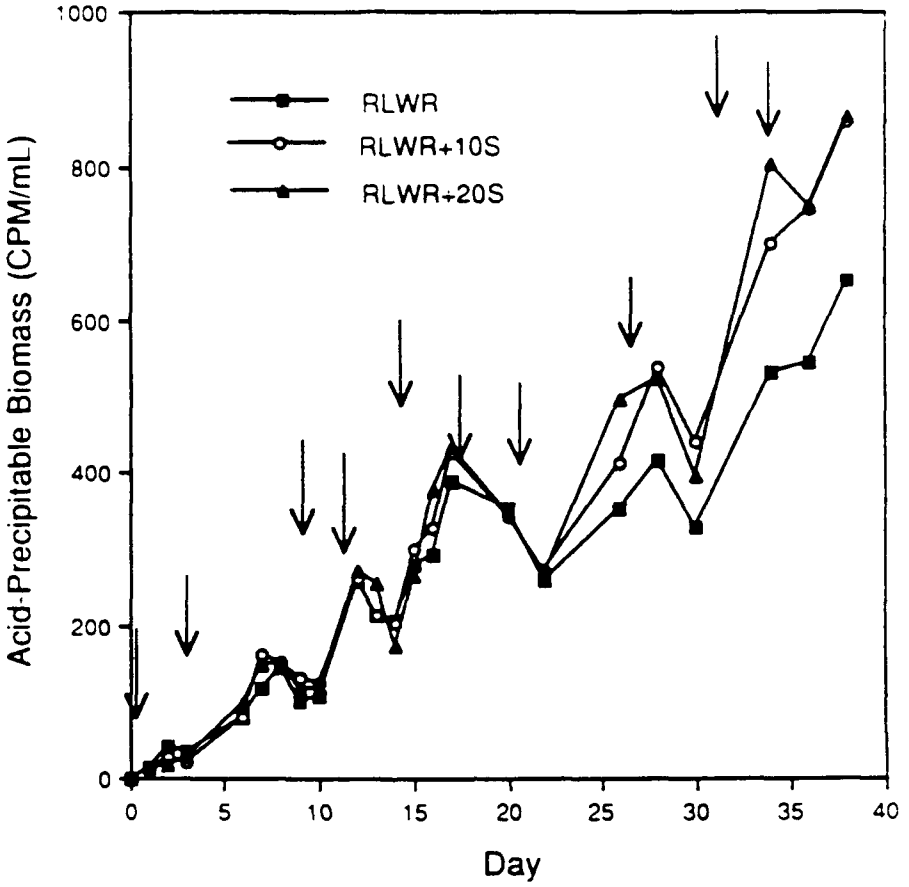

FIGURE 5 Conversion of ^{14}C-TNT to Biomass with Repeated Additions of Succinate (shown by arrows) (Key to flask designations: RLWR = RLWR consortium + succinate + 100 ppm ^{14}C-TNT, RLWR+10S = same as RLWR with 10 µL of succinate added every 3 d, and RLWR+20S = same as RLWR with 20 µL of succinate added every 3 d.)

indicating mineralization of the TNT. The total amount mineralized by day 48 was approximately 2.5%. This experiment is still in progress.

To determine if a similar result could be obtained with soil slurries, four 20% (weight to volume) RLWR soil slurries were made and placed in respirometers. All slurries contained approximately 140 ppm TNT, 0.1% succinate, and ^{14}C-TNT in Stanier's medium. The first slurry was inoculated with RLWR consortium, the second was inoculated with killed cells, the third was autoclaved RLWR soil with no added consortium, and the fourth was RLWR soil only. Again, the evolved CO_2 was trapped by KOH, which was sampled, replaced, and counted approximately every 2 d. The results of this experiment are shown in Fig. 7. The lower activity of the slurry containing soil and killed cells was probably due to the release of the cells' contents, which would have two possible effects. The cells contents could become toxic during autoclaving, which would inhibit biological activity, or, more likely, the cell contents and debris would provide an alternative carbon source, which would inhibit TNT conversion.

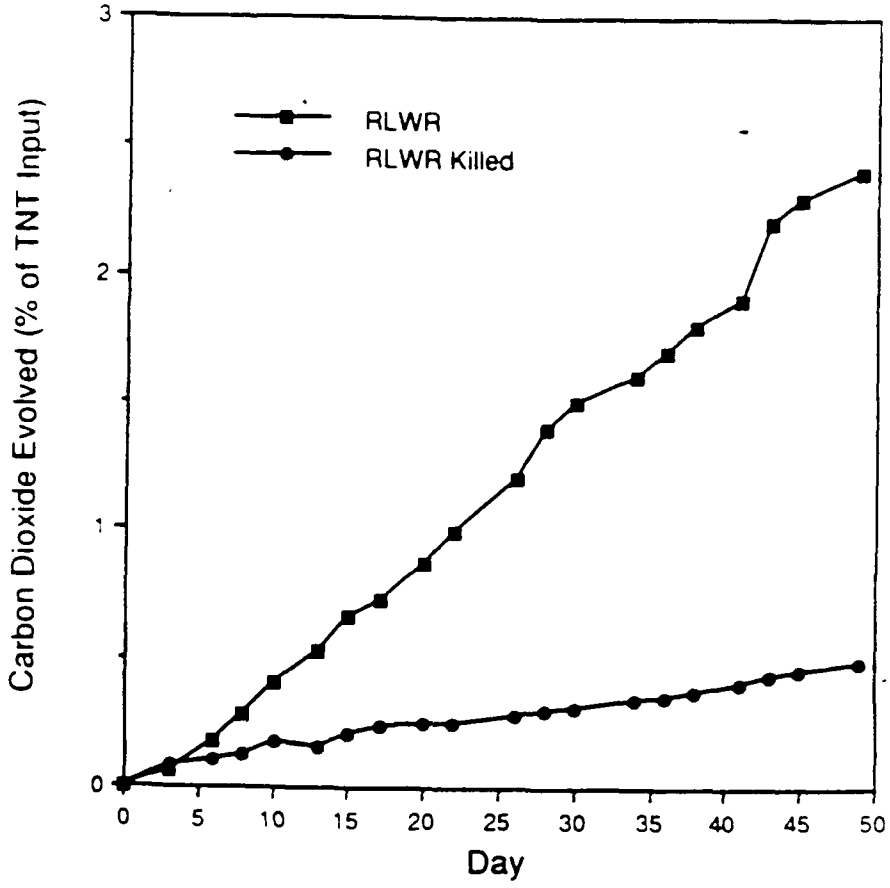

FIGURE 6 Evolution of ^{14}C-CO_2 from ^{14}C-TNT by RLWR Consortium

This experiment is still in progress, but the early data indicate that TNT is being mineralized to CO_2 in soil slurries. The RLWR-consortium sample shows the most metabolic activity, but significant activity is present in the soil-only sample. This activity could be due to the addition of succinate to the soils, which stimulated the indigenous organisms that were the source of the RLWR consortium. The requirement of succinate for metabolism in soil slurries will be determined in future experiments.

3.2 BENCH-SCALE REACTOR STUDIES

The soil used for the reactor studies was collected from washout area #2 (G6W2). As shown in Table 1, the TNT level in this soil was measured between 35,000 and 40,000 ppm. Comparable results of 34,000-38,000 ppm TNT were obtained at the University of Notre Dame.

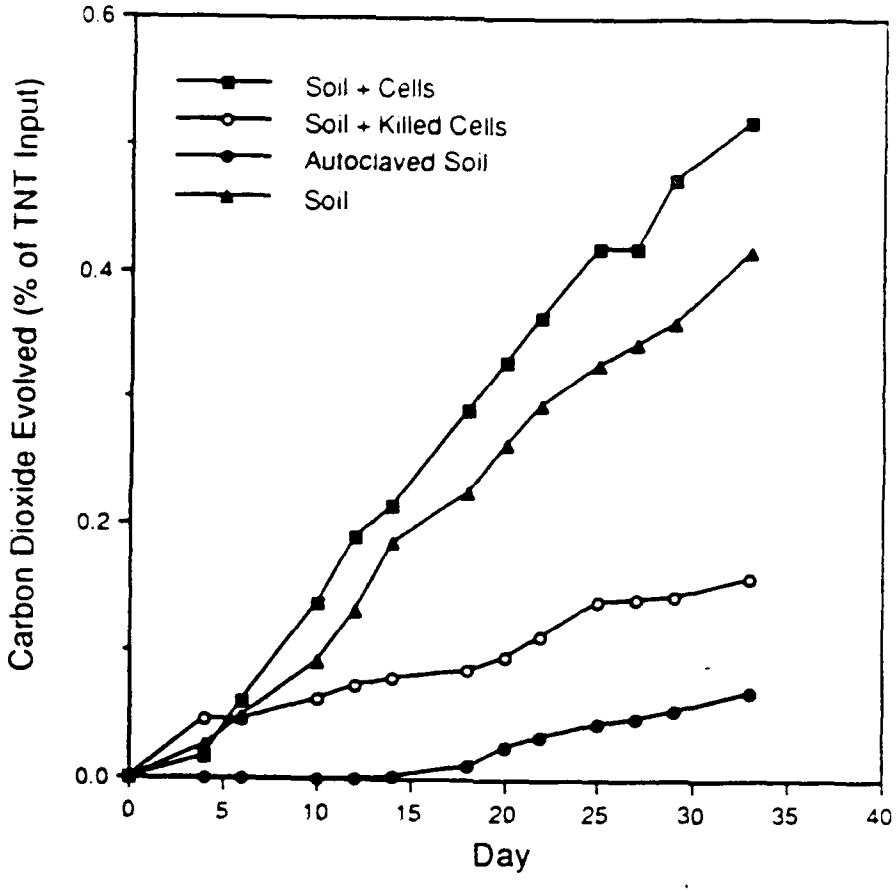

FIGURE 7 Production of CO_2 from ^{14}C-TNT by RLWR Consortium

3.2.1 Preliminary Slurry Reactor Evaluation

A 4-L glass kettle slurry reactor containing 200 mg of soil and 200 mL of tap water was vigorously agitated for 6 wk. The control for this experiment, which was quiescently maintained in the refrigerator until extraction, contained approximately 37,700 ppm TNT. After 6 wk of agitation, the soil from the slurry reactor contained 12,400 ppm TNT and the water from the reactor contained 96 mg/L TNT, an overall TNT reduction of 67%.

A second 4-L slurry reactor was then started with 2 L of tap water and 2 kg of soil and operated for 51 d. After 29 d, 47% of the TNT was removed. No appreciable removal of TNT was observed during the remainder of the test period. Subsequent measurement of available NH_4^+ indicated that the ion had decreased from 12 mg/L to less than 1 mg/L during the course of the study, suggesting that NH_4^+ may have have been limiting in the system.

3.2.2 Large-Scale Slurry Reactor Evaluation

A 10.9-L stainless-steel slurry reactor was constructed from a steel drum that was placed on its side. The reactor has a rotating shaft down the center with tines at 1-in. intervals that come to within 0.25 in. of the inside surface of the drum. The lower half of the drum has five rows of 0.25-in. square pegs that protrude approximately 0.25 in. between the blades to provide added agitation. The bottom row of pegs have holes for aeration. The top of the reactor has an access port for air exit, sampling, and soil and nutrient addition. In the reactor, 2 kg of the soil were added to 2 L of tap water. The initial TNT concentration was approximately 37,700 ppm. The slurry was mixed and aerated with water-saturated air continuously for 6 wk; samples were taken at 3 and 6 wk. Make-up water was added every 2 or 3 d to compensate for evaporative losses.

After 3 wk, the TNT concentration was reduced by about 68% to 11,900 ppm in the middle of the reactor, where the slurry was being vigorously mixed. About 5-10% of the soil had caked around the pegs on the bottom of the reactor, at the front and back, and on the side pegs. This caked soil had higher levels of TNT than the vigorously mixed soil in the middle of the reactor; the side, front, and bottom had 16,800, 19,000, and 31,100 ppm TNT, respectively. The reactor water contained 92 mg/L TNT. After 6 wk, a similar pattern was observed. The TNT concentration in the middle of the reactor was 8,900 ppm, and 15,200, 18,800, and 24,900 ppm, respectively, were measured in the cake at the side, front, and bottom. The water in the reactor contained 90 mg/L TNT. Because of the problems associated with mixing, blades were attached to the end of the tines to scrape the inner wall of the drum. Studies will be resumed when techniques to enhance the degradation are obtained from the 1-L reactors (Sec. 3.2.3).

Measurements were also made for oxygen uptake rate, total Kjeldahl nitrogen (TKN), ammonia, and phosphate. Samples from the reactor had shown an oxygen uptake rate only after the addition of both ammonia and phosphate. The TKN of the soil was 1.4 ppm. The phosphate content of the soils could not be determined. The soil became very orange when mixed with water, possibly due to the precipitation of the phosphate with iron in the soil. As a result, assays developed on the ion chromatograph for phosphate, ammonium, nitrite, nitrate, chloride, and sulphate ions were used for future determinations of these ions in the slurries and soils.

Variations in TNT concentrations were traced to the extraction procedure for TNT analysis. For example, results depended on where the sample was placed in the sonication bath. In addition, Soxhlet extraction resulted in about one-fifth as much recovery of TNT from the samples as the sonication bath, a wrist-action shaker resulted in the extraction of 20% more TNT than the sonication bath in just 5 min, and submersion of a sonicator horn in the sample resulted in the extraction of less TNT than the sonication bath.

As a result, several other extraction procedures, including use of a sonicator cup horn, surfactants, and other solvents, were investigated. To date, the best extraction method involves the use of the wrist-action shaker. Results from the sonicator cup horn are comparable to the wrist-action shaker; however, sample throughput with the horn is much greater than that for the wrist-action shaker. Replicate results for TNT analysis

on samples collected from each reactor at a given time agree within about 4%. Day-to-day variations for the same reactor, however, are quite high. This likely reflects difficulties associated with obtaining a representative sample from soil slurry reactors.

The HPLC procedure works very well; the chromatogram peak for TNT appears at 7 min±15 s. For the slurry reactor samples, a large peak has appeared at about 3 min. This peak becomes more prominent with increasing time of treatment. There are also several smaller peaks that appear before the TNT peak.

3.2.3 Initial Optimization of TNT Removal

Optimization studies involved the use of five 1-L reactors mixed by overhead stirrers. Various water-to-soil ratios were tested in these reactors to determine the best conditions for mixing and separation, to determine nutrient requirements, and to test the effect of succinate addition on the removal of TNT.

Mixing and settling studies indicated the best slurries were produced by a 10-20% (weight to volume) ratio of soil to water. These levels also facilitated the extractions and oxygen uptake measurements. The slurry concentration used in each of five 1-L reactors was 15% weight to volume. The first reactor was seeded with a 10% slurry from the second 4-L slurry reactor described above. The second reactor was a live control with nothing added. The third reactor had nutrient addition of NH_4^+ and PO_4^{+3} at 1 mg/L. Ten mg/L of succinate was added twice, once after a few days of operation and once after the oxygen uptake rate in the reactor subsided. The fourth reactor contained 2,000 mg/L succinate and the above nutrients. The fifth reactor was a dead control with the biological activity removed by autoclaving the slurry for 1 h at 124°C.

The third and fourth reactors were operated to test the effect of succinate on TNT removal. In the third reactor, no oxygen uptake was observed until after succinate was added. One day after succinate addition, the oxygen uptake rate increased to 4 mg/L·h, which then subsided 2 d later. At this point, 10 mg/L of succinate was added to a slurry sample in the Oxygraph (the instrument used to measure oxygen uptake rate). Based on the Oxygraph result, succinate was again added at 10 mg/L to the slurry reactor. The same pattern described above was again observed. This was the last addition of succinate to this reactor. Nutrients were added weekly.

One day after adding 1,000 mg/L succinate to the fourth reactor, the oxygen uptake rate increased to 4 mg/L·h. Additional nutrients brought this up to 6 mg/L·h. As a result, nutrients and an additional 1,000 mg/L succinate were added to this reactor. The oxygen uptake rate remained high for several weeks, varying between 6 and 8 mg/L·h. No more succinate was added.

The initial TNT concentration in the dead control, the fifth reactor, was substantially lower than that in the other reactors. The melting point of TNT is 82°C, well below the autoclave temperature of 124°C. This, plus the possible breakdown of the TNT or a tighter binding of TNT to the soil, reduced the measured value of TNT. There were, however, no significant differences seen in the HPLC chromatograms of samples collected from the dead control as compared to samples collected from the other reactors.

The only reactor that demonstrated a significant decrease in TNT was the fourth reactor, i.e., the one with the elevated levels of succinate. The TNT level decreased by approximately 40% during the first 24 d and then remained at this level through day 38 (the last day reported herein). The first, or seeded, reactor showed a decrease of approximately 15% through day 24 and also leveled off. The other reactors did not demonstrate any decrease in TNT levels. These results confirm that the bioremediation of TNT will likely require cometabolism with an alternate carbon source such as succinate. The more concentrated 50% slurries showed significant TNT destruction without the addition of succinate. These higher-concentration slurries may offer some conditions that promote the growth of organisms that remove TNT and should be studied further.

3.2.4 Removal of TNT by White-Rot Fungus

Prior to investigating the breakdown of TNT by the white-rot fungus, *Phanerochaete chrysosporium*, in a fixed-film reactor, the activity of pure ligninase alone on TNT was tested. A test was run with a buffer, hydrogen peroxide, and TNT at 100 mg/L. Ligninase was added to one of two flasks and not to the other (the control). After 1.5 h, there was a 32% reduction in TNT in the flask that contained ligninase as compared to the control.

A fixed-film reactor was established for growing white-rot fungus. After the fungus reached secondary metabolism, TNT was added to the reactor at an initial concentration of 83 mg/L. Samples collected every 3 d for 15 d showed that the concentration of TNT in the reactor decreased to 1.9 mg/L, with most of the TNT removed during the first 3 d. This preliminary evaluation of the white-rot fungus system shows that it holds promise and merits further evaluation.

4 DESIGN AND COST ANALYSIS OF AN SS/SBR SYSTEM

Based on the results reported above, a treatment system consisting of five SS/SBR tanks or reactors was designed. Each reactor was sized such that 250 yd^3 of soil can be slurried in each reactor with four times its volume in water. The number of individuals in the excavation crew was selected such that, each day for 5 d each week, 500 yd^3 of contaminated soil would be moved, 250 yd^3 from a reactor after treatment and 250 yd^3 into a reactor for treatment. The well, pumps, tanks, and mixers are designed to handle this capacity.

The reactors are designed to run on a 7-d cycle, allowing for draw and fill on the first day, followed by 5 d of reaction and 1 d for settling. The 24-hr settling period allows for at least a 50% recycle of the water. A 6-in. layer of slurry will be left in the bottom of the tank for seeding the next cycle, and 1.5 ft of freeboard will remain in the tank. Each of the five reactors will be drained and filled once per week. The reactor scheduled for draining and filling on Monday will require a timer to shut down the mixers on Sunday for settling. Two operators will be needed to operate the equipment, and one manager will be needed to oversee the operation and provide assistance. The excavation equipment will be rented with operators. Tank dimensions are based on a low profile and large surface area to aid in material input; the larger surface area will also increase aeration.

4.1 DESIGN DESCRIPTION

Figure 8 is an overhead view of the design. The excavation equipment consists of two dump trucks to transport soil from the point of excavation to the reactors then back again from the drying beds. Two front-end loaders are required to excavate the soil, to load the soil into the slurrying equipment, to remove the soil from the drying beds, and to return treated soil to the point of excavation. A grader is required to spread the soil on the drying beds and level it when it is returned to the point of excavation.

The soil slurrying equipment includes an aggregate screen with a shaker and water wash that empties the slurry over the tank. The aggregates are removed by shaking. The screen is attached to a soil elevator. The soil elevator supports the screen over the tank and feeds soil to the screen. A hopper is attached over the bottom of the elevator for loading soil. The elevator sits on a trailer for movement from tank to tank. The production capacity of the elevator and screen are up to 4 yd^3/min.

Each SS/SBR tank is 55 ft in diameter and 16 ft high (approximately 1,250 yd^3 capacity with 1.5 ft of freeboard to prevent overflow and to provide room for microorganism seeding between cycles). The tanks are made of fiberglass reinforced with steel bands. When empty, the tanks can be moved on site by crane. The tanks also can be disassembled and transported to another site.

The mixers are long-shaft-propeller, pit mixers that can be attached to the side walls or placed on floats. The shaft mixer is attached to its support point by a pivot that allows it freedom to swing through an adjustable arc using its mixing force. There are

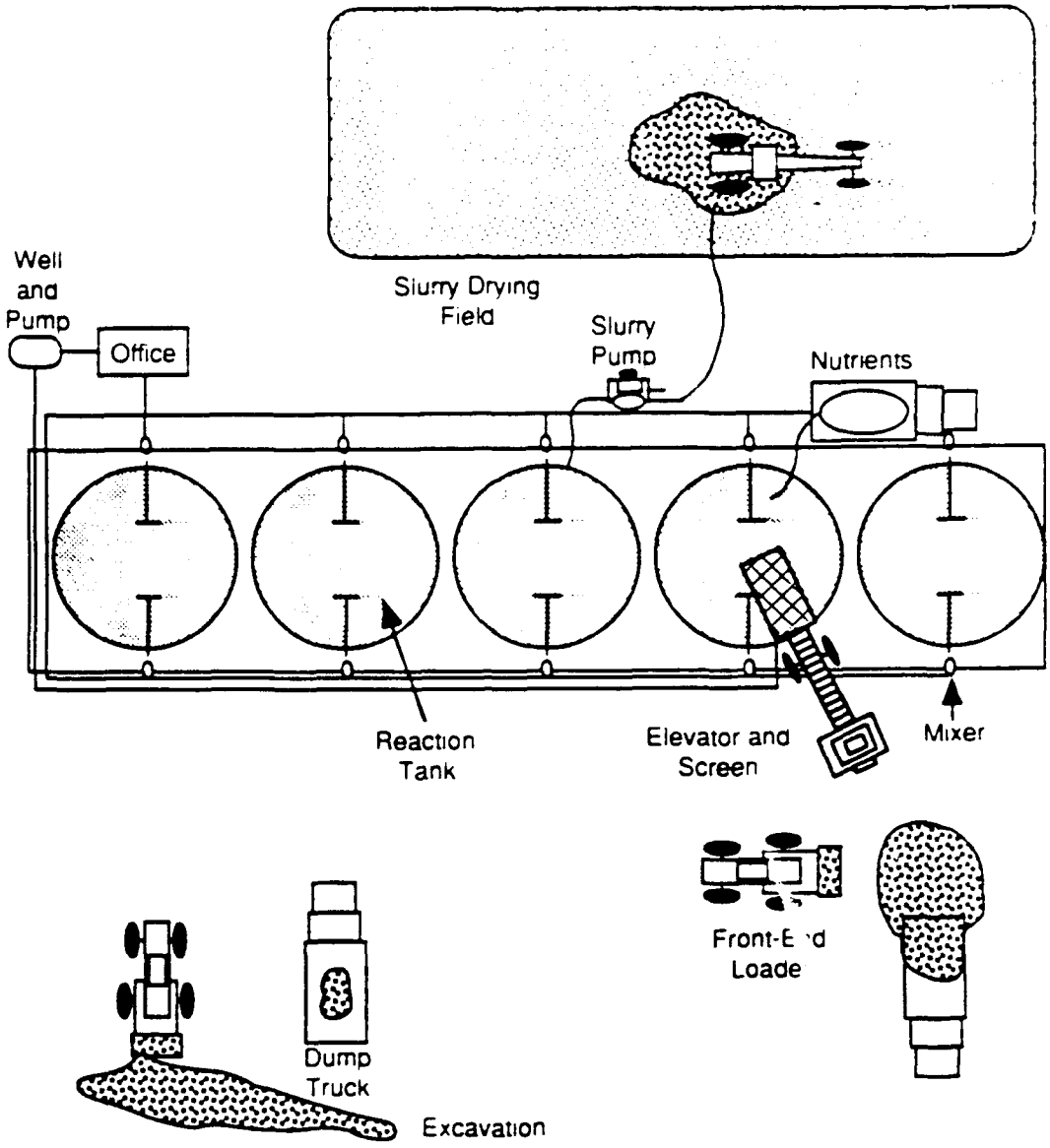

FIGURE 8 Conceptual Design of an SS/SBR Bioremediation System

two 20-hp mixers per SS/SBR tank. Because mixing is critical to the operation, two spare mixers are included in the design.

The slurry pump is portable and engine driven. It has the capacity to pump 1,000 gal/min at 25 ft of head and has a suction lift of 15 ft at 1,000 gal/min. This pump can be moved from tank to tank for daily operation and can be used to pump the contents of lagoons into the reactors. The pump will also be used to clean out the bottom of the tank when necessary. Because the entire operation centers around the slurry pump, a spare was added to the design along with necessary seals and rotors.

The well and pump for water supply are priced to cover a well 500 ft deep with a capacity of 500 gal/min, which should be adequate for most locations. The electrical

hookup for the well provides the connection box for the electrical requirements for the remainder of the site. Plumbing is provided through flexible hoses and manually operated gate valves. A 4-in. hose is used to plumb the tanks and the slurry screen. A 6-in., abrasion-resistant suction hose is used for the suction side of the slurry pump, and a 6-in. hose is used for the discharge side. Ball-and-socket connectors are used throughout the plumbing to provide the needed flexibility.

Nutrient delivery will be provided in liquid form on a daily basis from a local agricultural chemical supplier. Nitrogen and phosphate prices are built into the operating costs based on a carbon, nitrogen, and phosphate balance with TNT. Table 2 provides a component list for the equipment and structures for the design.

4.2 SYSTEM COSTS

4.2.1 Operating Costs

Table 3 provides an overview of cost estimates for the operating costs. Labor costs were based on a 40-h work week for each of two operators and one manager. Manpower requirements were based on equipment operation, routine maintenance, and equipment adjustment. The cost for the rental of the excavation equipment -- two 15-yd^3 dump trucks, two 3-yd^3 front-end loaders, and one grader -- includes operators and fuel.

Fuel costs are based on a $1.00/gal at an 8-h/d usage. Nutrient prices were obtained from a local agricultural chemical supplier and are based on solution delivered to the site.

4.2.2 Capital Costs

Table 3 also lists the initial capital costs for the SS/SBR tanks. These tanks can be salvaged at one-half the original cost after each relocation. The concrete work, site well, and electrical equipment are assumed to have no salvage value. All equipment -- office, fuel tank, pumps, mixers, plumbing, and slurrying equipment -- is considered to have a 2-yr operating life. Salvage on this equipment is the fraction of post-treatment operating life times the cost. The salvage value is subtracted from the initial cost of the item to obtain the cost of the item while used on the site.

4.2.3 Cost Estimates per Site Size

Table 4 provides cost estimates for three different quantities of soil: 10,000, 40,000, and 80,000 yd^3. Startup and shutdown times for all sites are estimated at 4 wk and include the use of an excavating crew 20% of the time. Thus, the total cost for startup and shutdown is $70,800.

TABLE 2 Component Quantities, Specifications, and Costs for a Five Reactor SS/SBR Bioremediation System

Component and Function	Quantity	Specifications	Cost ($)		
			Unit	Labor	Total
Concrete pad - base for reaction tanks	1	Dimensions are 350 ft x 75 ft x 6 in., reinforced with steel to bear 1 ton/ft^2	29,000	55,000	84,000
Concrete retaining wall - spill containment	1	Dimensions are 852 ft x 6 ft x 8 in., sealant for wall and pad	12,000	30,000	42,000
Well and pump - water supply	1	Well is 500 ft deep and has 500-gal/min capacity, pump is 40 hp	-	-	28,000
Electrical supply - for pumps, mixers, and office	1	Three-phase wiring for well pump and mixers, hookup for site office	-	-	16,000
Reaction tanks	5	Dimensions are 54-ft inner diameter and 16-ft height, capacity is 1,250 yd^3 with 1.5-ft freeboard, steel-reinforced fiberglass	172,000	-	860,000
Screen and elevator - to move and screen soil and add water	1	8- x 10-ft triple-deck screen, 2- x 40-ft elevator with 9-yd hopper and 16.6-ft drop, capacity is 250 gal/min and 3-4 yd^3/min	-	-	51,000
Slurry mixers - to mix and aerate soil	12[a]	14-ft, pivoting-shaft, propeller mixer; wall attachment plates; 20-hp, 3-phase motor	6,237	-	34,752
Slurry pump - to move soil from tanks to drying beds	2[a]	6-in. handling pump for 3-in.-dia. solids, silicone carbide impeller and seals, gasoline-fueled 150-hp engine, capacity is 1,000 gal/min with 20-ft head, trailer mounted	17,376	-	34,752

TABLE 2 (Cont'd)

Component and Function	Quantity	Specifications	Cost ($) Unit	Labor	Total
Plumbing - to connect pumps and water supply	400 ft	6-in. abrasion-resistant suction hose	16	-	6,400
	30	6-in. ball-and-socket connectors	96	-	2,880
	5	6-in. gate valves	47	-	235
	100 ft	6-in. discharge hose	7	-	700
	1,200 ft	4-in. suction hose	8	-	9,600
	12	4-in. ball-and socket connectors	54	-	648
	5	4-in. gate valves	36	-	180
	5	Floats	400	-	2,000
		Total cost for plumbing			22,643
Site office - for personnel and site management	1	10- x 30-ft mobile trailer; contains changing and cleaning area, storage for personal protection gear, break area, restroom, and office; has special shower room with large holding tank	-	-	20,000
Fuel tank - to store gasoline for slurry pump	1	1,000-gal aboveground tank	-	-	2,000

[a]Includes spares (two extra mixers and one extra pump).

TABLE 3 Design Costs for an SS/SBR System Using Excavation Equipment

Cost Item	Cost ($) Hourly	Weekly
Operating Costs		
Soil Excavation		
Two 15-yd^3 dump trucks	110	
Two 3-yd^3 front-end loaders	170	
One grader	85	
Total	365	14,600
Operators		
Supervisor	50	
Two laborers	70	
Total	120	4,800
Other		
Fuel (300 gal/wk x $1/gal)		300
Electricity (27,000 kWh/wk x $0.07/kWh)		1,890
Nutrients		1,350
Total		3,540
Total operating costs		22,940
During startup or shutdown		11,260

	Cost ($)	Salvage Value ($)
Capital Costs		
Components		
Concrete foundation and pad	84,000	0
Concrete retaining wall	42,000	0
Well and pump	28,000	0
Electrical	16,000	0
Reaction tanks	860,000	430,000
Screen and elevator	51,000	a
Mixers	74,844	a
Slurry pumps	34,732	a
Plumbing	22,643	a
Mobile office	20,000	a
Fuel tank	2,000	a
Subtotal	1,235,219	-
Contingencies at 10 %	123,522	0
Total capital costs	1,358,741	-

[a]Salvage value = cost x (104-weeks used)/104 wk. A 2-yr life is assumed.

TABLE 4 Cost Estimates for Selected Site Sizes Using Excavation Equipment

Cost Item	Site-Specific Costs ($)		
	10,000 yd^3, 12-wk Cleanup	40,000 yd^3, 36-wk Cleanup	80,000 yc^3, 68-wk Cleanup
Capital Costs			
Components			
Concrete foundation and pad	84,000	84,000	84,000
Concrete retaining wall	42,000	42,000	42,000
Well and pump	28,000	28,000	28,000
Electrical	16,000	16,000	16,000
Reaction tanks	430,000	430,000	430,000
Screen and elevator	5,885	17,654	33,346
Mixers	8,636	25,908	48,936
Slurry pumps	4,008	12,023	22,709
Plumbing	2,613	7,838	14,805
Mobile office	2,308	6,923	13,077
Fuel tank	231	692	1,308
Subtotal	623,679	671,037	734,182
Contingencies	123,522	123,522	123,522
Total capital costs	747,201	794,559	857,704
Operating Costs			
Startup and shutdown, 4 wk	45,040	45,040	45,040
Normal operation at $22,940/wk	183,520	734,080	1,468,160
Total operating cost	228,560	779,120	1,513,200
Total cost per site	975,761	1,573,679	2,370,904

The normal operating costs per site are based on the number of cubic yards at the site, divided by 1,250 yd^3/wk to obtain the total number of weeks required to process the soil. The number of weeks multiplied times the normal daily operating costs was added to the startup and shutdown costs to yield the total operating costs for each site.

Capital costs are based on the total capital and equipment costs less appropriate salvage values. This figure is added to the total operating costs to obtain a total cost per site. The total cost per site is divided by the cubic yards of soil processed to determine the costs per cubic yard. The cost per cubic yard was also calculated for extended treatment times of 2, 4, and 8 wk (Table 5). The extended operating times increased the costs linearly, the highest being $155/$yd^3$ at 8 wk to process 1,250 yd^3 at the 10,000-yd^3 site.

TABLE 5 Effect of Cycle Duration on Site-Specific Costs

Time to Treat 1,250 yd^3	Site-Specific Cost ($/$yd^3$)		
	10,000 yd^3	40,000 yd^3	80,000 yd^3
1 week	98	39	30
2 weeks	106	48	38
4 weeks	122	64	54
8 weeks	155	97	81

4.3 ALTERNATIVE SYSTEM COMPONENTS

Laboratory results have indicated that oxygen transfer rates of 6 mg/L·h can be expected. Assuming a COD equivalent for TNT of 1.06 mg/L and a total reaction time of 136 h, a slurry with a TNT concentration of 770 mg/L or less can be treated during a 1-wk cycle. Additional oxygen could be made available using conventional aeration equipment, using a pure oxygen atmosphere above the slurry, or adding hydrogen peroxide to the reactor if a higher oxygen transfer rate were desired. Longer or shorter reaction times could also be run, depending on the concentration of explosives in the reactor. The kinetics for TNT removal and completeness of treatment have not yet been defined. Alternative carbon sources such as succinate may be required for the reactors. This requirement has not yet been established. Such carbon sources are expected to be relatively inexpensive in bulk and should not add much to the operating costs.

If more rapid treatment is required, multiples of the five-tank SS/SBR system could be easily used and the size of each reactor in the system can be increased. The cost is also influenced by the type of tank used. For example, the cost of each steel-reinforced fiberglass tank is $172,000. The corresponding cost for a concrete tank is approximately $80,000. A concrete tank would not, of course, be portable, and the cost of disposal of the tanks would have to be considered. It should be noted that lined lagoons designed to the size required are also possible.

As is shown in Fig. 9, it may be possible to replace the excavating equipment used in the above cost analysis with a hydromining apparatus that uses water to displace, screen, and slurry the soil. In order to put the cost of such a system into perspective, a maximum cost estimate was obtained for hydromining equipment capable of handling 3-4 yd^3 of soil per minute. An estimate of $200,000 was provided by North American Machining, a manufacturer of these systems. As can be seen from a comparison of Tables 3 and 6, the extra capital cost associated with the hydromining equipment (versus

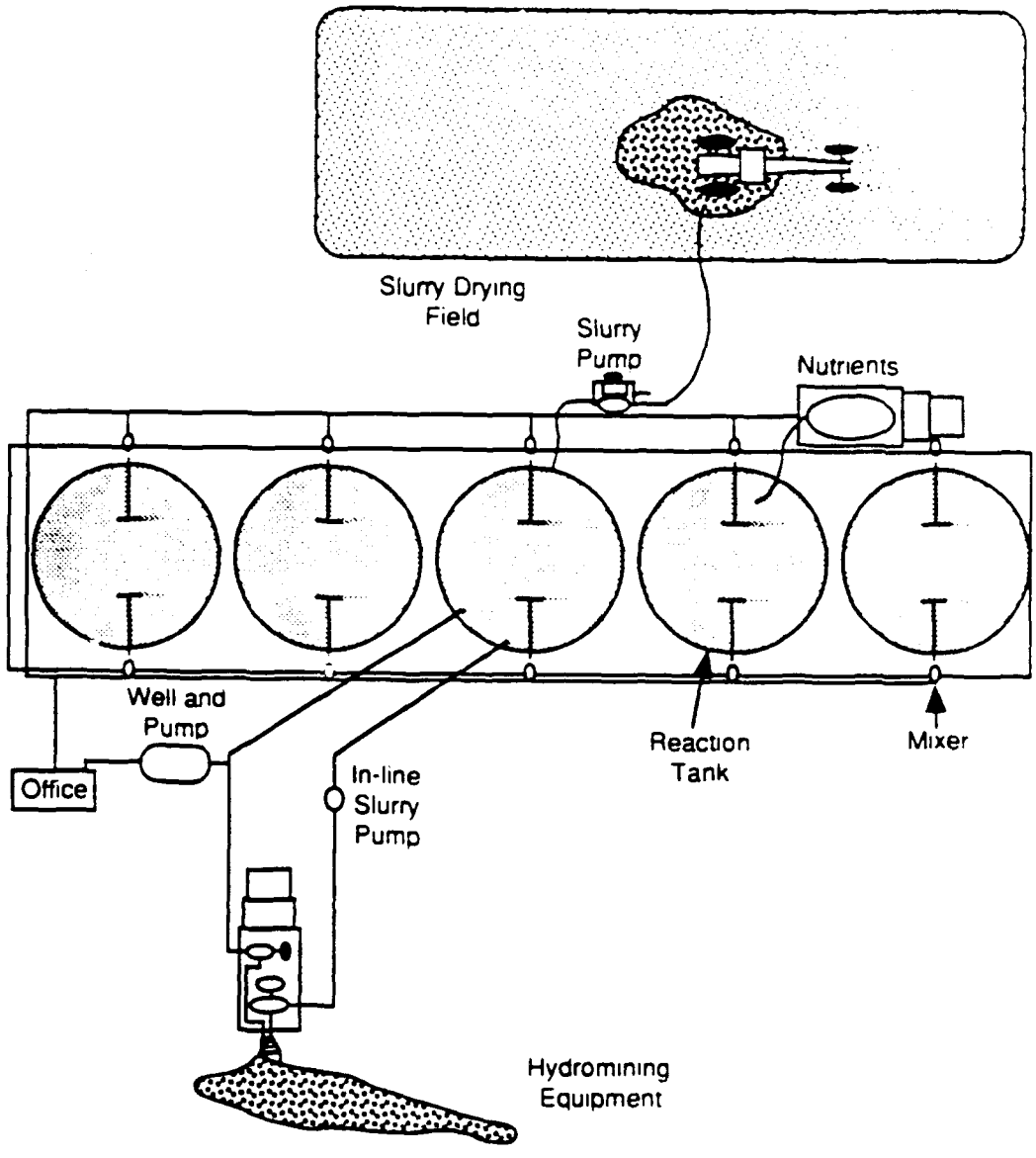

FIGURE 9 Conceptual Design of an SS/SBR Bioremediation System Using Hydromining Equipment

the excavating equipment) is easily recovered through reduced operating costs. A comparison of Tables 4 and 7 reveals that the cost per cubic yard for a 1-wk total cycle time is roughly $10/yd^3 less for the hydromining system than for the excavation system for all three site sizes. Additional advantages of the hydromining system include less handling and exposure to the contaminated soil and the ability to clean and leave large objects in place.

TABLE 6 Design Costs for an SS/SBR System Using Hydromining Equipment

Cost Item	Cost ($) Hourly	Weekly
Operating Costs		
Operators		
Supervisor	50	
3-Laborers	105	
Total	155	6,200
Other		
Fuel (300 gal/wk x $1/gal)		300
Electricity (27,000 kWh/wk x $0.07/kWh)		1,890
Nutrients		1,350
Total		3,540
Total operating costs		9,740
During startup or shutdown		9,740

	Cost ($)	Salvage Value ($)
Capital Costs		
Components		
Concrete foundation and pad	84,000	0
Concrete retaining wall	42,000	0
Well and pump	28,000	0
Electrical	16,000	0
Reaction tanks	860,000	430,000
Hydromining	200,000	a
Mixers	74,844	a
Slurry pumps	34,732	a
Plumbing	22,643	a
Mobile office	20,000	a
Fuel tank	2,000	a
Total	1,384,219	-
Contingencies at 10%	138,422	0
Total capital costs	1,522,641	-

[a]Salvage value = cost x (104-weeks used)/104 wk. A 2-yr life is assumed.

TABLE 7 Cost Estimates for Selected Site Sizes Using Hydromining Equipment

Cost Item	Site-Specific Costs ($)		
	10,000 yd^3, 12-wk Cleanup	40,000 yd^3, 36-wk Cleanup	80,000 yd^3, 68-wk Cleanup
Capital Costs			
Components			
Concrete foundation and pad	84,000	84,000	84,000
Concrete retaining wall	42,000	42,000	42,000
Well and pump	28,000	28,000	28,000
Electrical	16,000	16,000	16,000
Reaction tanks	430,000	430,000	430,000
Hydromining equipment	23,077	69,231	130,769
Mixers	8,636	25,908	48,936
Slurry pumps	4,008	12,023	22,709
Plumbing	2,613	7,838	14,805
Mobile office	2,308	6,923	13,077
Fuel tank	231	692	1,308
Subtotal	640,871	722,614	831,605
Contingencies	123,522	123,522	123,522
Total capital costs	764,393	846,136	955,127
Operating Costs			
Startup and shutdown, 4 wk	38,960	38,960	38,960
Normal operation at $9,740/wk	77,920	311,680	623,360
Total operating cost	116,880	350,640	662,320
Total cost per site	896,173	1,211,676	1,632,347
Cost per cubic yard	90	30	20

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APPENDIX A

EXPLOSIVES ANALYSIS PROCEDURE

Method No. LW02

EXPLOSIVES IN SOIL

I. SUMMARY

A. Analytes:

HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
RDX	Hexahydro-1,3,5-trinitro-s-triazine
NB	Nitrobenzene
1,3-DNB	1,3-Dinitrobenzene
1,3,5-TNB	1,3,5-Trinitrobenzene
2,4-DNT	2,4-Dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
2,4,6-TNT	2,4,6-Trinitrotoluene
Tetryl	2,4,6-Trinitrophenylmethylnitramine

B. Matrix: Soil or sediment

C. General Method: An aliquot of soil is extracted with acetonitrile. The acetonitrile is diluted with methanol and water, and the resultant solution is injected onto the HPLC for analysis.

II. APPLICATION

A. Tested Concentration Range:

HMX	1.27-140 ug/g
RDX	0.98- 80.0 ug/g
NB	0.42- 60.0 ug/g
1,3-DNB	0.59- 60.0 ug/g
1,3,5-TNB	2.09- 60.0 ug/g
2,4-DNT	0.42- 60.0 ug/g
2,6-DNT	0.40- 60.0 ug/g
2,4,6-TNT	1.92-100.0 ug/g
Tetryl	0.32- 24.9 ug/g

B. Sensitivity:

Peak Height in mm at an Attenuation of 2^4

HMX	48 mm for 14 ug/g
RDX	48 mm for 8.0 ug/g
NB	26 mm for 6.0 ug/g
1,3-DNB	53 mm for 6.0 ug/g
1,3,5-TNB	44 mm for 6.0 ug/g
2,4-DNT	31 mm for 10.0 ug/g
2,6-DNT	17 mm for 6.0 ug/g
2,4,6-TNT	45 mm for 6.0 ug/g
Tetryl	26 mm for 8.0 ug/g

C. Detection Limits:

HMX	1.27 ug/g
RDX	0.98 ug/g
NB	0.42 ug/g
1,3-DNB	0.59 ug/g
1,3,5-TNB	2.09 ug/g
2,4-DNT	0.42 ug/g
2,6-DNT	0.40 ug/g
2,4,6-TNT	1.92 ug/g
Tetryl	0.32 ug/g

D. Interferences:

1. Any compound that is extracted from soil that gives a retention time similar to the nitro-compounds and absorbs at 250 nm.

E. Analysis Rate:

After instrument calibration, one analyst can analyze two samples in one hour. One analyst can conduct sample preparation at a rate of three samples per hour. One analyst doing both sample preparation and the HPLC analysis can run 16 samples in an 8-hour day.

F. Safety information:

Work in well-ventilated areas. Wear adequate protective clothing to avoid skin contact. Wash skin with soap and water thoroughly immediately after contact.

TNB, HMX, RDX, Tetryl, and TNT's are classified as Explosives A by DOT. Avoid extreme temperatures and pressures.

Method No. DW02

III. APPARATUS AND CHEMICALS

A. Glassware/Hardware

1. Syringes: 10 uL, 50 uL, 100 uL, 1 mL syringe (Hamilton 1005 TEFLE)

2. Vials with Teflon-lined caps or septa. Nominal volume of 1.8 m L, 4.0 mL and 8.0 mL.

3. B-D Glaspak disposable syringes, 5 mLs, with frosted tip

4. 0.2 micron fluorocarbon filters

5. Micropipettes, 200 uL

6. Hypo needles

7. 2 mL. pipette

B. Instrumentation

1. Perkin-Elmer Series 4 High Performance Liquid Chromatograph (HPLC) equipped with a Perkin-Elmer ISS100 Auto-Injector and Micromeritics Model 786 UV/VIS variable wavelength detector. Hewlett-Packard 3390 recording integrator in peak height mode was used to record data output. ISS 100 auto injector is equipped with a temperature controlled sample tray jto refrigerate extracts.

2. Analytical Balance

   Capable of weighing 0.01 grams for sample preparation and 0.1 mg for standard preparation. Mettler AE 163 or equivalent.

3. Parameters

   a. Columns:

      1) DuPont Zorbax^{R} ODS 4.6 mm i.d. x 25 cm HPLC column with a particle size of 5-6 microns.

      2) DuPont Permaphase^{R} ODS guard column. (optional)

b. Mobile Phase: The water/methanol ratio must be adjusted as described in the calibration Section V.A.5.c to obtain optimum peak separation.

44-55% water
28-34% methanol
16-22% acetonitrile

c. Flow: 1.6 mL/min with a pressure of approximately 2860 psig.

d. Detector: 250 nm

e. Injection Volume: 50 uL

f. Retention Times:

	Minutes
HMX	3.30- 3.60
RDX	4.55- 4.70
NB	7.95- 9.00
1,3-DNB	7.30- 8.00
1,3,5-TNB	6.35- 6.40
2,4-DNT	11.00-13.10
2,6-DNT	10.60-12.40
2,4,6-TNT	10.05-10.90
Tetryl	9.15- 9.70

C. Analytes

1. Chemical Abstracts Registry Numbers

HMX	2691-41-0
RDX	121-82-4
NB	98-95-3
1,3-DNB	99-65-01
1,3,5-TNB	99-35-4
2,4-DNT	121-14-2
2,6-DNT	606-20-2
2,4,6-TNT	118-96-7
Tetryl	35572-78-2

2. Chemical Reactions

a. RDX and HMX can undergo alkaline hydrolysis.

b. RDX and HMX degrade at temperatures greater than 80_C in an organic solvent.

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3. Physical Properties

	Formula	Mol. Wt.	M.P. (°C)	B.P. (°C)
HMX	$C_4H_8N_8O_8$	296.6	276	-
RDX	$C_3H_6N_6O_6$	222.12	205	-
NB	$C_6H_5NO_2$	123.11	6	211
1,3-DNB	$C_6H_4N_2O_4$	168.11	90	302
1,3,5-TNB	$C_6H_3N_3O_6$	213.11	122	315
2,4-DNT	$C_7H_6N_2O_4$	182.14	71	300 (decomposes)
2,6-DNT	$C_7H_6N_2O_4$	182.14	66	-
2,4,6-TNT	$C_7H_5N_3O_6$	227.13	82	240 (decomposes)
Tetryl	$C_7H_5N_5O_8$	287.15	131	187

D. Reagents and SARMs:

1. Acetonitrile, distilled in glass for HPLC use
2. Methanol, distilled in glass for HPLC use
3. Water, distilled in glass for HPLC use
4. USATHAMA Standard Soil
5. SARMs

HMX SARM No. 1217(PA 1303)
RDX SARM No. 1130(PA 1302)
NB SARM No. (PA 1306)
1,3-DNB SARM No. 2250(PA 1305)
1,3,5-TNB SARM No. 1154(PA 1300)
2,4-DNT SARM No. 1147(PA 1298)
2,6-DNT SARM No. 1148(PA 1299)
2,4,6-TNT SARM No. 1129(PA 1297)
Tetryl SARM No. 1149(PA 1301)

IV. CALIBRATION

A. Initial Calibration

1. Preparation of Standards:

a. Stock calibration solutions containing approximately 10,000 mg/L of a nitro-compound are prepared by accurately weighing ca. 50 mg of a SARM into a 5 mL serum bottle and dissolving the nitro-compound in 5 mL of acetonitrile pipetted into the bottle. All stock solutions prepared in this manner and stored in a freezer (0°C to -4° C) have remained stable for a period of 6 months.

b. Intermediate Calibration Standards: All compounds appear to be stable for at least 3 months.

1) Intermediate Calibration Standard A (high level): Combine the appropriate volumes of stock calibration standard as shown below. Dilute to 5 mL with acetonitrile and seal with a Teflon-lined cap. Store in the dark at 0°-4°C. The resulting solution will have the concentrations indicated in the following table.

Nitro-compound	uL of Stock Cal Std	Resulting concentration (ug/mL)
HMX	175	350
RDX	100	200
NB	75	150
1,3-DNB	75	150
1,3,5-TNB	75	150
2,4-DNT	75	150
2,6-DNT	75	150
2,4,6-TNT	125	250
Tetryl	100	200

2) Intermediate Calibration Standard B (low level): 1:10 dilution of the Intermediate Calibration Standard A is made in Acetonitrile. Seal with a Teflon-lined cap and store in the dark at 0°-4°C. The resulting solution will have the following concentrations:

Nitro-Compound	Resulting conc. (ug/mL)
HMX	35.0
RDX	20.0
NB	15.0
1,3-DNB	15.0
1,3,5-TNB	15.0
2,4-DNT	15.0
2,6-DNT	15.0
2,4,6-TNT	25.0
Tetryl	20.0

c. Working Calibration Standards: Using the following table, prepare a series of ten calibration standards. Place the mobile phase into a 1-mL serum vial. Inject the indicated volumes of intermediate calibration standard A or B into the acetonitrile with a microliter syringe. Seal the vial with a teflon-lined septum and cap. Mix well. These solutions are prepared fresh daily and kept in the dark.

WORKING CALIBRATION STANDARDS

Conc.	Amt. (uL) Interned. Cal. Std. to Add A	B	Amt. (uL) Mobile Phase to Add	Resulting Concentration (ug/L) HMX	2,4,6-TNT	Tetryl RDX	1,3-DNB 1,3,5-TNB 2,6-DNT 2,4-DNT
0	0	0	2.0	-	-	-	-
0.2 X	-	1.0	999.0	35	25	20	15
0.5 X	-	2.5	997.5	87.5	62.5	50	37.5
1 X	-	5	995.0	175	125	100	75
2 X	-	10	990.0	350	250	200	150
5 X	-	25	975.0	875	625	500	375
10 X	5	-	995.0	1750	1250	1000	750
20 X	10	-	990.0	3500	2500	2500	1500
50 X	25	-	975.0	8750	6250	5000	3750
100 X	50	-	950.0	17500	12500	10000	7500

2. Instrument Calibration

   a. Set up the instrument according to the manufacturer's recommendations.

   b. Mobile Phase is analyzed as a blank to verify a stable baseline.

   c. Analyze the medium calibration standard (10X) to verify peak separation and retention times.

   d. Analyze the calibration standards prepared in Section IV.A.1.

3. Analysis of Calibration Data

   a. Tabulate the calibration standard concentration versus the peak height response for each calibration standard.

   b. Perform a linear regression analysis on the calibration data plotting peak height vs. concentration in ug/l.

4. Calibration Checks

   a. After completion of analyses of samples, a calibration standard at the highest concentration is analyzed. The response must agree within 25% for that concentration from the first seven calibration curves. Thereafter, the response must agree within two standard deviations of the mean response for that concentration. If it does not, the calibration standard will be reanalyzed. If the calibration standard fails this test, initial calibration must be performed, and all samples analyzed since the last acceptable calibration must be reanalyzed.

   b. No certified calibration check standards are available for these compounds.

B. Daily Calibration

1. Prior to analyses each day, a high calibration standard will be analyzed. For the first seven determinations at this concentration, the response must agree within 25% of the mean of all previous responses. After seven determinations, the response must agree within +/- two standard deviations of the mean response for previous determinations at this concentration.

2. If the calibration standard fails this test, it will be reanalyzed. If the calibration standard fails the second test, the system will have failed daily calibration, and initial calibration will be performed.

3. After completion of sample analyses each day, the high calibration standard will be analyzed again. The response for this calibration standard will be subjected to the criteria discussed in Section IV.B.1, above. If the response fails the criteria, the standard will be reanalyzed. If the second response fails the test, the system will have failed calibration, and initial calibration will be performed. All samples analyzed since the last acceptable calibration must be reanalyzed.

V. Certification Testing

A. Control Spikes:

To a series of ten 5-mL serum vials, approximately one gram of soil is accurately weighed into each vial. Using a syringe, the volumes of intermediate calibration standard indicated in the following table are injected onto the soil. The serum vial is covered with a septum and shaken until the soil no longer looks wet (approximately 60 seconds). The sample must equilibrate at least one hour. The septum is removed and the indicated amount (see Table below) of acetonitrile is pipetted onto the soil. The septum is replaced and the vial is capped. The sealed sample is shaken by hand for approximately 2-3 minutes. The sample is prepared via the procedure given in this method, to give the target concentrations in the following table.

Method No. LW02

CONTROL SPIKES

Conc.	Amt. (uL) Intermed. Cal. Std. to Add A	Amt. (uL) Aceto Nitrile to Add B	Resulting Concentration (ug/g) HMX	2,4,6 TNT	Tetryl RDX	1,3-DNB 1,3,5-TNB 2,6-DNT 2,6-DNT NB
0	0	2000	0	0	0	0
0.2 X	-	8.0	1992	0.28	0.2	0.16	0.12
0.5 X	-	20	1980	0.70	0.5	0.4	0.3
1 X	4	-	1996	1.40	1.0	0.8	0.6
2 X	8	-	1992	2.80	2.0	1.6	1.2
5 X	20	-	1980	7.0	5.0	4.0	3.0
10 X	40	-	1960	14.0	10.0	8.0	6.0
20 X	80	-	1920	28.0	20.0	16.0	12.0
50 X	200	-	1800	70.0	50.0	40.0	30.0
100 X	400	-	1600	140.0	100.0	80.0	60.0

VI. SAMPLE HANDLING STORAGE

A. Sampling Procedure: The stability of explosives in soil is not truly known. Precautions should be taken to avoid prolonged exposure to light and heat.

B. Containers: Wide-mouth amber glass bottles with teflon-lined lids.

C. Storage Conditions: Samples should be maintained at 4_ C from the time of collection to the time of analysis. No chemical preservatives are necessary.

D. Holding Time Limits: 7 days to extraction; 40 days to analysis from the time of extraction.

E. Solution Verification: No certified check standards are available.

VII. PROCEDURE

A. Separations

1. Accurately weigh 1 gram of soil into a 5-mL serum vial and pipette 2 mL of acetonitrile onto the soil.

2. Place a septum and cap on the vial and shake the vial thoroughly by hand for 2-3 minutes.

3. The extract is then filtered using the following technique.

   A 5-mL syringe is fitted with a needle. After the extract is drawn into the syringe barrel, a Fluorocarbon 0.2 micron disposable filter is attached in place of the needle. The sample is then slowly forced through the filter into a 4.0 mL teflon capped vial and stored until the extract is diluted and analyzed by HPLC. (Step 4-C.)

4. Preparation of sample extracts and spikes for injection is performed the day of analysis.

   a. Using a disposable micropipette, accurately measure 200 uL of filtered extract into a 1-mL vial. Accurately measure 600 uL of a 33% methanol/67% water solution onto the filtered sample. This will produce 800 uL of extracted sample in mobile phase.

   b. Place a septum cap on the vial. Shake the vial well to thoroughly mix. Store in the dark at 0°-4° C until ready to analyze.

B. Chemical Reactions - None. Compounds are read directly.

C. Instrumental Analysis:

1. Set the chromatographic conditions as follows:

	Time (minutes)	Flow (mLs/min.)	MeCN %	MeOH %	HOH %
Equilibrium	2	1.6	16	34	50
Analysis Run	20	1.6	16	34	50

2. All standards and extracts should be in chilled tray (4° C)

3. Using the auto-injector manufacturer's recommended procedure, introduce 50 uL of the medium level calibration standard into the

chromatographic system. Check the chromatogram to ensure separation of the nitrated toluenes and separation of the nitrobenzene and tetryl. If necessary, adjust the water/ methanol ratio of the mobile phase until separate peaks are distinguished. As the column ages, less methanol is required. Generally, the column ages rapidly the first 24 hours, after which it is fairly stable.

4) Once good peak separation is obtained, introduce 50 uL of each working calibration standard and sample into the chromatographic system using the auto-injector manufacturer's recommended procedure.

VIII. CALCULATIONS

A. The diluted extract concentration is read or calculated from the instrument calibration curve.

B. Sample Concentration (ug/g) = extract conc X $\frac{B \times D}{A \times C}$

where:

A = sample weight (dry weight)

B = mL acetonitrile used to extract sample

C = mL acetonitrile extract diluted into mobile phase

D = final volume in mL of mobile phase prepared for injection

NOTE: When samples are prepared according to this method (1 gram extracted into 8 mL of mobile phase), the above calculation becomes:

Sample Concentration (ug/g) = extract conc (ug/l) X 0.008

IX. DAILY QUALITY CONTROL

A. Control Samples

1. Intermediate Spiking Standard A and B are made according to Section IV just as calibration standards.

2. Daily control samples are prepared in a manner identical to that described in Section V. A total of three control spikes are required on a daily basis: two at 10X and one at 2X. They will have the following concentrations.

Conc.	Amt (uL) Intermed. Spiking A to add to 2.0 mls Acetonitrile	HMX	2,4,6-TNT	Tetryl RDX	2,4-DNT 2,6-DNT 1,3-DNB 1,3,5-TNB NB
2X	8	2.8	2.0	1.6	1.2
10X	40	14.0	10.0	8.0	6.0

3. At least one method blank using the USATHAMA Standard Soil is also analyzed with each analytical lot.

4. At least one matrix spike (actual sample) at 10X is analyzed for each analytical lot or at a frequency of 10%, whichever is more frequent.

B. Control Charts:

1. Average Percent Recovery (X)

a. Percent recoveries for the 10X certification spikes from days 1 and 2 are averaged to obtain the first value to be plotted.

b. Percent recoveries for the 10X certification spikes from days 3 and 4 are averaged to obtained the second value to be plotted.

c. Percent recoveries for the method spikes closest to the certification 10X concentration from the first day of analyses are averaged to obtain the third value to be plotted.

d. Values from a, b, and c are averaged to determine the central line of the control chart.

e. Differences in percent recoveries for each pair of values in a, b, and c are averaged to obtain R.

f. The upper and lower warning limits are +/- 1.25 R from the central line.

g. The upper and lower control limits are +/- 1.88 R from the central line.

2. Difference in percent recoveries (R)

a. The value for R obtained in Section IX.B.1.e, above, is the base line of the control chart.

b. The warning limit is 2.511 R.

c. The control limit is 3.267 R.

3. Three Point Moving Average X

a. The average percent recovery from the 5 ug/g concentration from the first three days of certification testing is the first point to be plotted.

b. Subsequent points to be plotted are the average percent recoveries from the 5 ug/g concentration from the next group of three determinations (e.g., certification days 2, 3, and 4; certification days 3 and 4 and the first day of analysis; certification day 4, day 1 of analysis, and day 2 of analysis; etc.)

c. The central point on the control chart is the average of the plotted points and changes with each added point.

d. The range for each point is the difference between the highest and lowest values in each group of three determinations. The average range (MAR) is used to define the warning and control limits.

e. The upper and lower warning limits are +/- 0.682 MAR, respectively.

f. The upper and lower control limits are +/- 1.023 MAR, respectively.

4. Three point Moving Average R:

a. The base line is the MAR.

b. The warning limit is 2.050 MAR.

c. The control limit is 2.575 MAR.

5. Certified Calibration Check Standard:

   a. If available, two certified calibration check standards are analyzed with samples.

   b. For the first 20 determinations, results must fall within the acceptable range specified by the source of the standard.

   c. After 20 determinations, the mean value of the 20 determinations is used as the central line of a control chart.

   d. Warning limits are +/- two standard deviations.

   e. Control limits are +/- three standard deviations.

X. REFERENCES

A. USATHAMA Method 2C Cyclotrimethylenetrinitramine (RDX) in Soil and Sediment Samples, 12-3-80.

B. USATHAMA Method 8H Explosives in Water by HPLC, 12-27-82.

XI. DATA

A. Off-the-Shelf Analytical Reference Materials Characterization: Not Applicable

B. Initial Calibration

1. Response versus concentration data: See attached.

2. Response versus concentration graphs: See attached.

3. LOF Tests: Not applicable.

4. II Tests: Not applicable.

C. Daily Calibration

1. Response: Not applicable.

2. Required percentage or two standard deviation limits: Not applicable.

D. Standard Certification Samples

1. Tabulation and graph of found versys target concentrations: See attached.

2. LOF and II tests for the pooled data: See attached.

3. Calculated least squares linear regression line, confidence bounds, reporting limit, accuracy, standard deviation, percent imprecision, and percent inaccuracy: See attached.

4. Chromatograms: Attached

FIGURE 1 Data: Colony Counts for JAAP Soil Samples

Sample	CFU/G Day 0	CFU/G Day 6
W28V	1.7E7	1.4E8
WO1E	2.1E4	4.6E6
RLRW	1.0E7	1.4E7
WO2S	1.2E6	3.6E7
RLD2	1.5E6	1.9E7
RLD1	5.6E5	3.6E6
G6W1	1.7E4	1.4E7
TPDT	8.536	6.2E7
RWLW	1.2E5	7.0E6
G6W2	1.2E6	1.1E7

FIGURE 2 Data: Microbal Growth Analysis of Selected JAAP Soils

Time	RLRW	TPDT	WO1E	G6W2
0	1.0E7	8.5E6	2.1E4	1.2E6
3	2.5E7	4.0E7	6.1E6	
6	1.4E7	6.2E7	4.6E6	1.1E7
9				6.9E6
10	1.7E7	7.5E7	2.2E7	
13	1.7E7	2.5E7	3.7E7	8.1E6
15	2.4E7	1.3E7	4.0E7	
17				8.4E6
18	7.2E7	3.5E7	1.8E7	
21	8.7E7	5.1E7	5.5E7	
22				9.0E6
27	2.8E8	4.2E7	6.2E7	

FIGURE 3 Data: Assessment of RLWK Consortium Effect on Total TNT Culture (CPM/ML)

DAY	RS+S	RS+SK	RS	S+S	% TNT RS+S	% TNT RS+SK	RS %	S+S%
0	18983.5	19766	19372	19409.7	100.000	100.000	100.000	100.000
1	17870-3	19425.3	19090.4	19269.7	94.100	98.300	98.500	99 300
2	18517.2	10952.7	18793.6	19079.7	97.500	96.400	97.000	98.300
3	18412.7	19333.9	18517.6	19000.8	97.000	97.800	95.600	97.900
4	18544.4	19317.9	18637	19066.6	97.700	97.700	96.200	98 200
5	18086.3	19079.4	18553.6	18945.9	95.300	96.500	95.800	97.600
6	18068.9	19399	19189.8	19270.1	95.200	98.100	99.100	99 300
7	18057.3	18996-9	18668.6	18878	95.100	96.100	96.400	97.300
8	18322.3	19056.3	18784.9	19299.1	96.500	96.400	97.000	99 400
9	17793.3	19456	18783.5	19073.3	93.700	98.400	97.000	98 300
12	18098.8	19099.3	18446.8	19424.8	95.300	86.600	95.200	100 100
13	18231.3	19382.7	18716	19198.1	96.000	98.100	96.600	98 900
14	18041.5	19609.1	18656.5	19241.9	95.000	99.200	96.300	99 100
15	10820-6	19293.1	18506.2	19167.9	94 900	97.600	95.500	98 800
16	18038.5	19617	18779.3	19222.6	95.000	99.200	96.900	99 000

FIGURE 4 Data: Conversion of ^{14}C-TNT to Biomass

DAY	RS+S	RS+SK	RS	S+S	RS+S%	RS+SK%	RS %	S+S%
0	3.5		3.5		0.018		0.018	
1	124.75	77.5	19.75	92.25*	0.657	0.392	0.102	0.475
2	240			253.5	1.300			
3	250.25	147.5	21.25	147.5	1.300	0.476	0.110	1.300
4	399	141	163.75*	28.25	2.100	0.713	0.845	0.760
5	545.75	50.25			2.900	0.254		0.145
6	417.25				2.200			
7	726.75	10	17.75	48.5	3.800	0.051	0.092	0.250
8	898.75				4.700			
9	1104.5				5.800			
12	1990.25	4	20	9.25	10.500	0.020	0.103	0.048
13	1749.25				9.200			
14	1486.5*	8*	26.75		7.800	0.040	0.138	
15	2093				11.000			
16	2422.75	1	36.25		12.800	5.059e-3	0.187	
19	2981.25							

FIGURE 5 Data: Conversion of ^{14}C-TNT to Biomass with Repeated Additions of Succinate (CPM/ML)

	DAY	RLRW A	RLRW B	RLRW + 10 A	RLRW + 10 B	RLRW + 25 A	RLRW + 25 B	RLRW	RLRW + 10S	RLRW + 25S
1	0	.5	1.25	•	•		•	0.875		
2	1	12.25	14.75	15.5	6.75	12	18	13.500	11.125	15 500
3	2	20.25	64	22	32	18.25	15.5	42.125	27.000	16 875
4	3	27.75	38.5	18.25	26	30	27.5	33.125	22.125	28.750
5	6	68.75	90.5	89	71.5	94	100.25*	79.625	80.250	97.125
6	7	118.5	113.5	194.75	132.75	142.75	154	116.000	163.750	148.375
7	8	132.5	155.25	174.25	127.75	143.25	163.25	143.875	151.000	153.250
8	9	62.75	135	126	135	98.75	136.25	98.875	130.500	117.500
9	10	112	100.75	148.75	98.5	99	136	106.375	123.625	117.500
10	12	256.75	259	287.5	237.5	234.25	309.75	257.875	262.500	272 000
11	13	197.25	231.75	247.75	179.75	240 5	270.75	214.500	213.750	255 625
12	14	194.25	218.75	231	178.25	186	157	206.500	204.625	171 500
13	15	240	322.25*	345.5	253.75	311	222.25	281.125	299.625	266 625
14	16	238.5	346.5	395	263.25	362.75	388.75	292.500	329.125	375 750
15	17	385.5	388.75	445.25	405.25	411.25*	452.5	387.125	425.250	431 875
16	20	361.5	341.25	370.25	312.75	340.5	350.25	351.375	341.500	345.275
17	22	254	267.75	368.75	179.25	275	259.75	260.875	274.000	267 375
18	26	406.75	299	410.5	412.5	501.25	485.75	352.875	411.500	493 500
19	28	436.25	390.75	588	482.5	499	545	413.500	535.250	522 000

FIGURE 6 Data: Evolution of ^{14}C-CO_2 from ^{14}C-TNT by RLWR Consortium (CPM/ML)

	Day	RLRW	RLRW KILLED
1	3	92.75	124
2	6	269.5	156.25
3	8	443.25	191.75
4	10	633.25	236.125
5	13	821.75	278.875
6	15	1028	314.125
7	17	1129.5	374.375
8	20	1355	376.375
9	22	1551	385.875
10	26	1898.75	425.625
11	28	2107.25	459.375

FIGURE 7 Data Production of CO_2 from ^{14}C-TNT by RLWK Consortium

DAY	RLRW + CELLS	R + CELLS KIL	RLRW KILLED	RLRW	R + CELLS	% CO2 R + CELLS K	R KILLED	RLRW
0	0	0	0	0	0.000	0.000	0.000	0.000
4	26	73	0	41	0.017	0.047	0.000	0 026
6	94	73	0	76.5	0.060	0.047	0 000	0 049
10	216	97	.5	144	0.139	0.062	3.207e-4	0.092
12	296.5	113.5	.5	205.5	0.190	0.073	3.207e-4	0.132
14	335	123.5	3.5	290	0.215	0.079	2.245e-3	0.186
18	452	132.5	17.5	353	0.290	0.085	0.011	0 226
20	511	149	39.5	410.5	0.328	0.096	0 025	0.263
22	566	176.5	52	458	0.363	0.113	0.033	0.294
25	651.5	218	67	508	0.418	0.140	0 043	0.326
27	651.5	221.5	76.5	535	0.418	0.142	0.049	0.343
29	737	335	86	562	0.473	0.144	0.055	0.360
33	807	247	107.5	650.5	0.518	0.158	0 069	0.417
35	816	247	107.5	664.5	0.523	0.158	0 069	0.426
40	8996.5	275.5	136	754.5	0.575	0.177	0.087	0.484
43	958	290.5	137	806.5	0.614	0.186	0.088	0.517
47	1064.5	322	153	858	0.683	0.207	0.098	0 550
50	1117	351.5	153	868.5	0.716	0.225	0.098	0 557
54	1142.5	361.5	153	889.5	0.733	0.232	0.098	0 571
57	1219.5	421.5	216.5	948.5	0.782	0.270	0 139	0 608
51	1297.5	487	234	1032.5	0.832	0.312	0.150	0 662